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Response to dietary carbohydrates in European seabass (Dicentrarchus labrax) in muscle by an NMR-based metabolomics approach

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Introduction: Feed optimization is a key step to both environmental and economic aquaculture sustainability, especially for carnivorous species. Plant-derived ingredients can contribute to reduce costs and nitrogenous effluents while sparing wild stocks. However, the metabolic use of carbohydrates from vegetable sources by carnivorous fish is still not completely understood.

Objectives: We aimed to study the effects of diets with carbohydrates of different digestibilities, gelatinized starch (DS) and raw starch (RS), in the muscle metabolome of European seabass (Dicentrarchus labrax).

Methodology: We followed an NMR-metabolomics approach, using two sample preparation procedures, the intact muscle (HRMAS) and the aqueous muscle extracts (1H NMR), to compare the variations in muscle metabolome between the two diets.

Results: Overall, in muscle, multivariate analysis revealed a similar response to DS and RS diets, when compared with the control diet. Preparation of aqueous extracts involved discarding the organic phase, however, through univariate analysis of intact muscle it was possible to identify a general lipid increase in the muscle of DS-fed fish. The combined results of the univariate analysis of intact muscle and aqueous extracts indicate specific diet related changes in lipid and amino acid metabolism.

Conclusions: Due to differential sample processing, outputs differ in detail but provide complementary information. From the two tested diets, DS seems to be the most promising alternative to the regular fishmeal diet in aquaculture.

The potential metabolic role of the bacterial community of fish gut and their prey in a euthrophic shallow lake

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Metagenome data was analyzed using PICRUSt (Langille et al. 2013) to determine the microbiome functions, which showed that fish gut and environmental compartments exhibited similar profiles of gene functions at level 1, including 1) metabolism, 49.7±1.0% (mean±SE); 2) genetic information processing, 16.1±0.25%; 3) unclassified gene functions, 14.4±0.20%; 4) environmental information processing, 13.1±0.64%; 5) human diseases, 1.3±0.04%; 6) cellular processes, 4.4±0.32%; 7) organismal systems, 0.7±0.02%.

The functional pathways in all analyzed groups were dominated by genes associated with cellular metabolism pathways, which increases in genes associated with carbohydrate, amino acid metabolism and membrane transport, and to a lesser extent pathways associated with signaling molecules and interaction, environmental adaptation and metabolic diseases. Significant differences were noted for twenty-four genes in functional potential from mucosa and content communities. No significant differences were detected except amino acid metabolism (environment was higher than prey at $p \leq 0.05$) between predicted functional potential for environment and prey communities. Also, we have identified differences in predicted functional metagenomic pathways for intestinal content of different feeding habit groups whereas for intestinal mucosa no differences were detected (an exception was metabolism of terpenoids and polyketides between OM and ZB-ZP, at $p \leq 0.1$). No significant differences were detected between gastric mucosa and agastric mucosa (ANOSIM, $p > 0.1$).A scatter plot based on PCoA scores (the input in % of every gene category in total for level 2) showing that functional pathways are strongly divided on bacteria that associated with content and mucosa for all studied fish. In both mucosa and content groups of bacteria the predicted functional potential of communities between the stomach and intestine was similar. No clear grouping in the functional pathways of prey and environment were observed.

The functional data obtained in this study demonstrate that the fish, prey and environmental microbiota were more similar with regard to carbohydrate, amino acid metabolism and membrane transport. This somewhat similar to studies which showed that functional pathways of rainbow trout, Oncorhynchus mykiss were associated with carbohydrate, protein and amino acid metabolism genes and to a lesser extent with energy, vitamin and lipid metabolism (Lyons et al., 2016).

Untargeted GC-MS Metabolomics Reveals Metabolic Differences in the Chinese mitten-hand crab (Eriocheir sinensis) fed with dietary palm oil or olive oil

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Palm oil (PO) and olive oil (OO) have been widely used in animal diets, but the knowledge of metabolic change in animals fed a diet with different oil sources is limited. Here we compared the metabolic differences in the Chinese mitten crab Eriocheir sinensis fed with PO and OO as the main dietary lipid sources (6% diet) by performing GC-MS-based serum metabolomics assays and traditional nutritional assessments. The crab fed OO displayed lower lipid accumulation and oxidative stress in the hapatopancreas than those in the crab fed PO. In the metabolomics assay, a total of 68 metabolites with high credibility were identified and five metabolites were significantly different between two feeding groups. In these five metabolites, hydroxylamine, 3-hydroxypropionic acid and 2-hydroxypyridine were higher in the OO group, while lysine and citrulline were higher in the PO group. After combining the nutritional data with metabolomics assays, we demonstrate that olive oil can provide comprehensive benefits to the crab by providing more energy, improving cell membrane structure, containing phenols as a natural antioxidant, and improving the composition of intestinal microbiota. Conversely, as compared with olive oil, palmitic acid-enriched palm oil tended to increase protein degradation and lipid accumulation-induced lipotoxicity. This study illustrates that the metabolic mechanism differs between crabs fed olive oil and palm oil, and OA-enriched olive oil is recommended as an ingredient for crab diet formulation. The findings of study can serve as a baseline reference for future research to understand lipid metabolism in crustaceans.

Growth, osmoregulation capacity and metabolomics analysis of the Pacific white shrimp (Litopenaeus vannamei) fed different lipid sources under two salinities

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The effect of fish oil containing highly unsaturated fatty acids and coconut oil containing saturated fatty acids on the growth, osmoregulatory capacity of the L. vannamei were investigated, and then a metabolomics assay was performed with the purpose of trying to reveal the underlying mechanism of different fatty acids in dealing with osmoregulation of L. vannamei. Tree purified diets with fish oil (FO), coconut oil (FO) or equal mixed oil with fish oil and coconut oil (MO), respectively, were fed to juvenile L. vannamei at salinity of 3(low salinity, L) or 30 (High salinity, H) for eight weeks. The six groups were named as LFO, HFO, LCO, HCO, LMO or HMO. Shrimp at salinity 30 had higher weight gain and survival than at salinity 3 regardless of dietary lipid sources. At low salinity 3, shrimp weight gain and survival, highly unsaturated fatty acids in shrimp gill and hepatopancreas tended to increase with increasing dietary fish oil. Na+/K+-ATPase and total ATPase activities decreased as the decrease of fish oil in salinity 30 group, but the activities in shrimp of LMO group were significantly lower than groups of LCO and LFO. On the contrary, the relative mRNA expression of Na+/K+-ATPase showed the opposite tendency as the enzyme activities. The metabolomics results showed that serine, tyrosine, oleic acid, glucose were significantly higher in LCO group than LFO group, and tyrosine, lysine, palmitic acid and oleic acid were significantly higher in HCO group than HFO group. In both LCO and LFO groups, shrimp serum tyrosine significantly increased when compared with the HCO and HFO groups, while serum glycine decreased. These results suggest that fish oil has a positive influence on shrimp osmoregulation, but proper saturated fatty acid supplementation with fish oil could pose a promotion effect on shrimp osmoregulation than single fish oil application. The regulation of dietary fatty acids on shrimp osmoregulation could be mediated through interfere the amino acid metabolism in shrimp, but further studies should be conducted on this topic because no information on the direct connection has been reported.

Metabolic response of Nile tilapia (Oreochromis niloticus) to acute and chronic hypoxia stress

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Hypoxia is a critical issue in aquaculture, especially in intensive aquaculture systems. Acute hypoxia stress with dissolved oxygen (DO) 0.6 mg/L ~ 0.8 mg/L for 6 h and chronic hypoxia stress with DO 1.0 mg/L~1.2 mg/L for 4 weeks were used to investigate the response of nutritional metabolic pathways in Nile tilapia Oreochromis niloticus. Fish in the acute and chronic experiments had different adaptive mechanisms. Upon acute hypoxia stress, the contents of liver glycogen and muscle glycogen were significantly lower than in the normoxia group, but there was no significant difference in triglycerides in serum, liver and muscle. Hexokinase and fructose phosphate kinase activities increased after exposure to acute hypoxia stress. The mRNA expression of genes involved in glycolysis and glycogenolysis was significantly up-regulated by acute hypoxia stress. However, the response of fish to long-term hypoxia stress was different from it to acute hypoxia. In the hypoxia group, the crude fat in whole fish and triglycerides in liver and muscle were significantly lower than that in the normoxia treatment. Beta oxidation of the liver was enhanced in the hypoxia group, while the hepatic glycogen content increased in the hypoxia group. Transcriptomic analysis showed that the expression of genes related to carbohydrate synthesis and lipolysis increased in the hypoxia group, while genes related to carbohydrate catabolism and fat synthesis showed the opposite. This study indicates that fish could utilize carbohydrate as a main energy source during acute hypoxia stress, and metabolize more lipid during long-term hypoxia stress. A high carbohydrate content in the diet may help reduce negative effects from acute hypoxia stress, and an appropriate increase of fat content in the diet may benefit fish growth in a hypoxia environment, e.g., in high-density aquaculture ponds.

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Transcriptomic response to different dietary soy lecithin levels in female Portunus trituberculatus

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A transcriptome library was constructed using lllumina HiSeq 4000 high-through sequencing based on the mix of female swimming crab hepatopancreas and ovarian. In our project, we generated about 6.73 Gb bases in total after Illumina Hiseq sequencing. Then assemble all samples together, we got 55,667 Unigenes, the total length, average length, N50, and GC content of Unigenes are 53,579,003 bp, 962 bp, 1,785 bp, and 46.46 %, respectively. And then annotate Unigenes with 7 functional databases, finally, 26,267 (NR: 47.19%), 21,351 (NT: 38.35%), 21,683 (Swissprot: 38.95%), 11,089 (COG: 19.92%), 20,903 (KEGG: 37.55%), 2,047 (GO: 3.68%), and 17,536 (Interpro: 31.50%) Unigenes are annotated. With functional annotation results, we detected 26,121 CDS, and after predected by ESTScan with the remaining Unigenes, we got 4,274 CDS more. We also detected 4,155 SSR distribute on 17,966 Unigenes. Several lipid metabolism and gonadal development related genes, such as delta-6 desaturase, delta-9 acyl-CoA desaturase, ELOVL4, fatty acid binding protein, fatty acid synthase, vitellogenin, vitellogenin receptor, cdc2, cyclin B and cyclin H were identified. Using high-fluxed sequencing technology, our results would provide a rich source of data to understand transcriptome information of Portunus trituberculatus discover and identify new genes, characterize gene expression and make the first step for further genomics researches. To obtain an overview of gene expression profile in female swimming crab with hepatopancreas and ovarian as well as different soy lecithin treatments. cDNA libraries were constructed from hepatopancreas and ovarian as well as 0% and 4 % soy lecithin treatment groups. Their hepatopancreas and ovarian were collected for RNA isolation, transcriptome sequencing and analysis. We sequenced six different hepatopancreas samples and six different ovarian samples of female Portunus trituberculatus species using RNA-Seq technology, averagely generating 24,137,878 raw sequencing reads and then 24,133,636 clean reads after filtering low quality, of which 90.9 % of the clean sequence were high-quality sequence and can be used for subsequent analysis. The average mapping ratio with reference gene is 84.63% and qRT-PCR validation results were consistent with RNA-seq data. 146 genes (103 up-regulated, 43 down-regulated) were characterized as the most differentially expressed genes between 0% and 4 % soy lecithin treatment

Combination regulation of transcriptomics and miRNA in fish oil oxidative stress and emodin protection in Megalobrama amblycephala

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This study was aimed to investigate the oxidized fish oil stress and emodin protection mechanism. Twelve weeks feeding trial was conducted with 6% fish oil (control, 6F), 6% oxidized fish oil (6OF), and emodin supplementation (30 mg/kg) groups (6F+E, 6OF+E) in Megalobrama amblycephala (initial body weight: 5.20±0.01g).Weight gain rate (WGR) in 6OF was significantly lower ($P < 0.05$) compare with 6F, plasma AST and GSH-Px showed the reverse pattern, while 6OF+E decreased the AST concentration ($P < 0.05$) than 6OF. The ultrastructure of M. amblycephala intestine cell and mucosae in 6OF displayed tissue damage, while 6OF+E was intended to attenuated compare to 6OF, which revealed the injury stress of oxidized stress and emodin protection in histomorphological level. RNA and miRNA sequencing analysis was combined to evaluate the regulatory interaction. Transcriptomics analysis revealed the 21942 different genes between 6F and 6OF group, including 11375 up-regulated and 10567 down-regulated. Among them, 60 genes were differentiated significantly with 26 up-regulated and 34 down-regulated genes, respectively. These significance of altered genes participated in biological processes of stimulus response, immune process, and metabolic regulation based on GO annotation. Meanwhile, KEGG pathway enrichment showed they mainly were involved in the pathways of graft−versus−host disease, intestinal immune network for IgA production, antigen processing and presentation, and inflammatory bowel disease. miRNA sequencing discovered 85, 288, and 283 significantly expressed miRNAs in 6OF, 6F+E, and 6OF+E group, respectively. Target genes of these miRNAs in 6OF played important roles in carbon metabolism, glycolysis, pentose phosphate pathway, thyroid hormone signaling pathway, PI3K-AKT, NF-κB and MAPK signaling pathway. Association analysis of transcriptomics and epigenetics revealed that the complex regulatory networks of p53, mTOR, Notch, T cell receptor, PI3K-AKT, NF-κB and MAPK signaling modulated oxidative stress and emodin protection mechanism.Taken together, these results provide new insights into the lipid oxidative stress and emodin protection by transcriptome and epigenetic research.

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Evaluating the immunostimulant effect of a experimental diet on gills mucosal tissue using transcriptomic tools: from gene expression to the biological context.

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The immunostimulant effects studies have been mainly focused on the nonspecific systemic response but less on their consequences on other sites in direct contact with the environment, such as the mucosa. The lack of information about the effects of immunostimulant diets on mucosal tissues urgently demands to generate knowledge that allows understanding of the immunosupplemented diets influence on these tissues. In this context, there is also limited available information addressing the modulation of immune system-related genes that does not allow a direct understanding of the possible pathways and immunological functions involved. Therefore, the aim of this study was to evaluate the gills transcriptomic response of gilthead sea bream (Sparus aurata) fed with functional diets using both molecular and cellular approaches. The experiment involved 360 healthy sea bream (BW 38±7.3 g) separated in 27 tanks and fed with three formulated diets (Skretting): a commercial immunostimulant diet (diet A), an experimental immunostimulant top-coated supplemented diet (diet B), and a commercial standard diet (diet C). Fish were fed at a feeding rate of 3% BW twice daily for 28 days. Gills were sampled at 2, 7, 14 and 28 days of feeding (dof) and analyzed by microarrays and in situ hybridization (ISH). In order to determine the transient changes in both functional diets using the control diet as universal reference time-point, a loop analysis was performed comparing each time-point in a progressive temporal timescale. Microarray results showed a differential expression of genes associated to immunological processes such as inflammation and cellular response, among others. A more marked upregulation on genes related to both processes was observed in diet B than diet A compared to diet C. These differences were mainly registered at 14 dof, although the intensity and magnitude of these responses was not high. On the other hand, ISH analysis showed localization of immunological transcripts in a specific cellular type at the primary lamellae of gilthead sea bream gills. Altogether, these results indicate that immunostimulant diets modulate the expression of immune-related genes on gills, opening the perspective to the use of these tools to understand the immunosupplemented diet influence on these tissues.

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Development of high-throughput proteomic resources for the Giant Tiger Prawn, Penaeus monodon, and implications for nutrition research.

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Proteomics has been successfully applied to understanding stress, disease and quality in aquaculture species, mainly salmon, trout and catfish. This technique has not been widely applied to aquaculture nutrition, often due to a lack of proteomic and genomic resources for non-model organisms. Most frequently the term proteomics, as it applies to aquaculture, means the separation of complex protein mixtures using 2D gel electrophoresis, followed by peptide fragment mapping of individual spots. Recent advances in the field of mass spectrometry (MS) have revolutionised proteomics, enabling simultaneous identification and quantification of hundreds of proteins from complex mixtures using peptide fragment fingerprinting.

In this study, we describe the development of a discovery proteomics pipeline from prawn haemolymph, hepatopancreas and muscle. A method was developed to remove haemocyanin, that constitutes up to 90% of haemolymph total protein, and enabled greater detection of proteins in haemolymph. Using a publicly available crustacean database (UniProt), 301, 131 and 193 proteins were detected from hemolymph, hepatopancreas and muscle tissue, respectively. Protein detection increased significantly to 612, 395, 682 hits within the same data set, respectively, when using a recently developed Penaeus monodon transcriptome through the ARC Hub for Advanced Prawn Breeding. Across these tissues, we successfully identified proteins of functional significance in prawn growth (myostatin, moult inhibiting hormone), immunity (prophenoloxidase, crustin, anti-lipopolysaccharide factor), antioxidant defence (peroxidases, glutathione-S transferase), stress (heat shock proteins) and metabolism (protein, lipid and carbohydrate). These proteins are currently being quantified in response to a range of dietary ingredients, or in response to stress, under controlled laboratory conditions. This study demonstrates that proteomics provides an efficient and powerful tool for discovery and quantification of novel proteins involved in diverse pathways, in response to dietary ingredients from non-model organisms.

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ISOLATION AND CHARACTERIZATION OF ELOVL5 AND ELOVL2 ELONGASES FROM TAMBAQUI (COLOSSOMA MACROPOMUM)

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Tambaqui (Colossoma macropomum) is a native fish species of the Amazon basin, being one of the main species in Brazilian aquaculture with a production of approximately 134ton in 2014. Therefore, it is important to determine nutritional requirements in this species to develop efficient and economical feeds. The biosynthetic capability of long-chain (C20) polyunsaturated fatty acids (LC-PUFA) including the biologically active eicosapentaenoic acid (EPA; 20:5n-3), arachidonic acid (ARA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3), has been extensively studied in many commercially important fish species to identify which fatty acids can satisfy the essential fatty acid (EFA) requirements for those species. LC-PUFA can be biosynthesized through the combined action of two enzymes, namely elongation of very long chain fatty acids (Elovl) proteins and fatty acyl desaturases. However, little is known about these LC-PUFA biosynthesizing enzymes in Tambaqui. Here we have aimed to isolate and functionally characterize putative Elovl5 and Elovl2 elongases from Tambaqui, enzymes with key roles in LC-PUFA biosynthesis of vertebrates. Phylogenetic analyses confirmed that the isolated elovl cDNAs are orthologs of Elovl5 and Elovl2. Moreover, functional characterization, carried out in a yeast heterologous expression system, revealed that the Tambaqui Elovl5 showed the ability to elongate C18 and C20 PUFA substrates, but no C22 substrates. Interestingly Elovl2 showed the ability to efficiently elongate PUFA substrates of varying chain lengths (C18, C20 and C22) and producing in some instances PUFA products of up to 26 carbons. While the capacity to elongate C18 and C20 PUFA substrates is shared with Elovl5, the Tambaqui Elovl2, unlike Elovl5, can also elongate 22:5n-3 to produce 24:5n-3, a key intermediate component of the so-called “Sprecher pathway”, a metabolic route that accounts for DHA biosynthesis in vertebrates. Given that complementary functions of the Tambaqui Elovl5 and Elovl2, the present results confirm that this species can perform all the elongation reactions enabling the conversions from C18 PUFA into physiologically important LC-PUFA (ARA, EPA and DHA). Importantly for aquaculture, such enzymatic capabilities strongly suggest that Tambaqui can efficiently utilize dietary oil sources such as vegetable oils rich in C18 PUFA to satisfy their EFA requirements.

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Hepatic glucose metabolic responses to digestible dietary carbohydrates in two isogenic lines of rainbow trout

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Rainbow trout (Oncorhynchus mykiss) was recognized as typical "glucose-intolerant" fish and poor dietary carbohydrate user. Our objective was to test the effect of dietary carbohydrates itself (without modification of dietary protein intake) on hepatic glucose gene expression (taking into account of the paralogs) and to compare this effect on two isogenic trout lines. We used two isogenic lines of rainbow trout (named A32h and AB1h) fed with either high carbohydrate diet or low carbohydrate diet for 12 weeks. We analysed the zootechnical parameters, the plasma metabolites, the hepatic glucose metabolism at a molecular level and the hormonal-nutrient sensing pathway. Globally dietary carbohydrate intake was associated with hyperglycemia and down regulation of the energy sensor Ampk, but also with atypical regulation of glycolysis and gluconeogenesis in liver. Indeed, the first steps of glycolysis and gluconeogenesis catalysed by the glucokinase and the phospenolpyruvate carboxykinase are regulated at the molecular level by dietary carbohydrates as expected (i.e. induction of the glycolytic gck and repression of the gluconeogenic pck); by contrast, and surprisingly, for two other key glycolytic enzymes (phosphofructokinase enzyme – pfkl – and pyruvate kinase – pk -) some of the paralogs (pfklb and pklr) are inhibited by carbohydrates whereas some of the genes coding gluconeogenic enzymes (the glucose-6-phosphatase enzyme –g6pcb1b and g6pcb2a gene and the fructose1-6 biphosphatase paralog fbp1a) are induced. On the other way, some differences for the zootechnical parameters and metabolic genes were found also between the two isogenic lines, confirming the existence of genetic polymorphisms for nutritional regulation of intermediary metabolism in rainbow trout. In conclusion, our study determines some new unexpected molecular regulation by dietary carbohydrates of the glucose metabolism in rainbow trout and underlines the existence of differences in molecular regulation of glucose metabolism between two isogenic lines.

Molecular characterization, tissue distribution of carnitine palmitoyltransferase genes and effects of dietary fish oil replacement on their expression in the heptatopancreas of Chinese mitten crab Eriocheir sinensis

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The carnitine palmitoyltransferase (CPT) is consists of two types of enzymes, CPT 1 and CPT 2, which are involved in the transport of long-chain fatty acids into the mitochondrial compartment for β-oxidation. In this study, three isoforms (CPT 1α, CPT 1β and CPT 2) of the CPT family were cloned from Chinese mitten crab (Eriocheir sinensis) and their complete coding sequences (CDS) were obtained. Sequence analysis showed that the proteins of three CPT isoforms contained 915, 775 and 683 amino acids respectively and were unstable protein without signal peptide and transmembrane region domain. Gene expression analysis via real-time quantitative polymerase chain reaction revealed that the three CPT isoforms widely distributed in various tissues, with high CPT 1α and CPT 2 expression levels in the hepatopancreas either from male or female, CPT 1β had high expression in muscle, heart (male) or ovary (females), and was more abundantly expressed in most tissues than other two isoforms. In order to investigate the characterization of the three CPT isoforms expression in hepatopancreas, a feeding experiment with different fish oil replacement levels (i.e. 0, 25%, 50%, 75% and 100%, defined as feed 1#, 2#, 3#, 4# and 5#, respectively) was conducted. The results showed that CPT 1α and CPT 2 expression level had the similar trend of first increased then decreased in the hepatopancreas among the five treatments, as well as CPT 1β of females; The CPT 1β expression level in hepatopancreas of males decreased with the increasing dietary fish oil replacement levels but without significant difference ($P > 0.05$) in males. These results indicated that hepatopancreas or muscle had the high expression of the genes related to fatty acid β-oxidation, and that dietary fish oil replacement had significant effects but the patterns were different on their expression, these suggested that they might play different roles in fatty acid β-oxidation.

Effects of the replacement of vegetable oil on the serum lipid metabolism in large yellow croaker (Larimichthys crocea)

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The study was conducted to investigate the effects of dietary vegetable oil on the serum lipid metabolism in large yellow croaker. Three diets were formulated by replacing 0% (FO), 50% (FV) and 100% (VO) fish oil with vegetable oil. Each diet was randomly fed to triplicate groups of fish for 10 weeks. Lipidomics change of L. crocea fed different diets were explored. Results showed that the serum lipid metabolite profiles were obviously different among three groups and a total of 39 lipid biomarkers were identified. Semi-quantification of individual lipid species in three groups were also conducted. Results demonstrated that the proportion of PC decreased, whereas TAG increased as FO gradually replaced by VO. To elucidate the mechanism by which dietary VO regulated serum PC and TAG levels, gene expression of key enzymes in hepatic and intestinal PC and TAG metabolism were further assessed. Compared with FO and FV group, VO group significantly inhibited the expression of hepatic CCTα, CPT and PEMT. However, no significant differences were detected in hepatic sPLA2 mRNA expression among the groups. The transcript levels of hepatic MTP and apoB100 were increased in FV and VO groups, but there were no obvious differences in DGAT2 expression. Besides, fish in FV and VO groups displayed higher expression of DGAT1 in intestine. An in vitro study was further performed to verify the results obtain in vivo. Results showed that DHA and EPA up-regulated the mRNA levels of CCTα, CPT in the primary hepatocytes. Compared with control group, fish in LA group displayed obvious lower PEMT mRNA expression. LA and ALA showed adverse regulation of DGAT2 mRNA levels. In addition, ALA group showed the most abundant expression of MTP and apoB100 levels. However, no significant differences in sPLA2 expression were detected among all treatments. In summary, these results demonstrated that dietary VO might regulate lipid composition and content, especially PC and TAG in serum by modulating hepatic and intestinal PC and TAG metabolism, which may be attribute to the change of fatty acids composition with the replacement of FO by the VO.

Life-stage associated remodeling of lipid metabolism regulation in Atlantic salmon

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Atlantic salmon migrates from rivers to sea to feed, grow and develop gonads before returning to spawn in freshwater. The transition to marine habitats is associated with dramatic changes in the environment, including water salinity, exposure to pathogens, and shift in dietary lipid availability. Many changes in physiology and metabolism occur across this life-stage transition, but little is known about the molecular nature of these changes.

Here we use a long term feeding experiment to study transcriptional regulation of lipid metabolism in Atlantic salmon gut and liver in both fresh- and saltwater. We find that lipid metabolism becomes significantly less plastic to differences in dietary lipid composition when salmon transitions to saltwater and experiences increased dietary lipid availability. Expression of genes in liver relating to lipogenesis and lipid transport decrease overall and become less responsive to diet, while genes for lipid uptake in gut become more highly expressed. Finally, analyses of evolutionary consequences of the salmonid specific whole-genome duplication on lipid metabolism reveals several pathways with significantly different duplicate retention or duplicate regulatory conservation. We also find a limited number of cases where the whole genome duplication has resulted in an increased gene dosage.

In conclusion, we find variable and pathway-specific effects of the salmonid genome duplication on lipid metabolism genes. A clear life-stage associated shift in lipid metabolism regulation is evident, and we hypothesize this to be, at least partly, driven by non-dietary factors such as the preparatory remodeling of gene regulation and physiology prior to sea migration.

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EFFECT OF PLANT-BASED DIETS WITH VARYING RATIOS OF ω6 TO ω3 FATTY ACIDS ON GROWTH, TISSUE COMPOSITION, HEPATIC GENE EXPRESSION AND FATTY ACID BIOSYNTHESIS IN ATLANTIC SALMON (SALMO SALAR)

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To date, inclusion of vegetable oils in aquafeeds has had little influence on fish performance. However, most vegetable oils provide unbalanced ratios of omega-6 to omega-3 (ω6:ω3) fatty acids (FAs) which have a crucial role in immune response and FA metabolism. There have been concerns that extensive replacement of fish oil with plant oil causes a high incidence of cardiovascular and inflammatory disorders in fish, and that farmed seafood may not supply an adequate ω6:ω3 ratio for human consumption. Although many studies have been conducted on the importance of this ratio for human health, very little is known about how variation in ω6:ω3 ratios affect FA metabolism and eicosanoid synthesis in salmon, and the potential underlying molecular mechanisms. Furthermore, the interaction between 18:2ω6 (LNA) and 18:3ω3 (ALA) remains unclear. The current study examined the impact of five plant-based diets (12-week exposure) with varying ω6 (soy oil) to ω3 (flaxseed oil) ratios (0.35-2.7) on the growth, lipid tissue composition and hepatic transcript expression of key lipid and eicosanoid synthesis genes (in 3 dietary groups using qPCR) in salmon. Growth performance and organ indices (HSI and VSI) were not affected by dietary ω6:ω3. The liver and muscle FA composition was highly reflective of the diet (ω6:ω3 of 0.2-0.8 and 0.3-1.9, respectively) and indicated elongation and desaturation of the ω3 and ω6 precursors. Compound-specific stable isotope analysis showed that the δ13C (13C/12C) signature of liver ARA was highly reflective of the dietary LNA in the fish fed with the high ω6 diet. This demonstrated significantly higher synthesis of ARA in the high ω6 compared to the high ω3 fed fish. Furthermore, the transcript levels of elovl2 and elovl5a were negatively correlated with EPA and the sum of ω3 FA, while lxra showed positive correlation with ALA and negative correlations with ARA and DHA in the liver. No statistically significant diet-induced effect was observed in the expression levels of fadsd5 and fadsd6. Finally, significant correlations were also identified between eicosanoid synthesis genes (e.g. pgds and cox1) and liver FAs. In summary, this study demonstrated the impact of dietary ω6:ω3 on LC-PUFA and eicosanoid synthesis.

A systemic study of lipid metabolism regulation in salmon larvae and early juvenile fed vegetable oil

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Compared to adults, salmon larvae and early juveniles often have immature lipid absorption, transport and biosynthesis systems, which can limit the sufficient utilization of a plant-based diet with high vegetable oil content. We carried out two experiments using lipid and transcriptomic analyses to present a systemic view of lipid metabolism regulation in salmon at larval and early juvenile stages. The first experiment compared the lipid metabolism in salmon larvae before and 20 hours after first feeding, aiming to understand the effect of first feeding on absorption, biosynthesis and transport pathways of different lipids. In pyloric caeca, the onset of an external diet caused a rapid up-regulation of phospholipid, cholesterol and chylomicron synthesis pathways and down-regulation of triacylglycerol synthesis pathway (Figure 1). This was associate with increased amounts of free fatty acids, monoacylglycerol and lyso-phospholipids and decreased amounts of phospholipid in pyloric caeca. In liver, the amount of triacylglycerol was decreased 50% after feeding, but the genes were less regulated. Based on these results, we suggest several bottlenecks for lipid absorption and transport in salmon larvae including insufficient intestinal phospholipid and chylomicron synthesis and hepatic bile acid excretion. These could reduce the efficient utilization of dietary lipids and growth in early stages of salmon. The second experiment compared lipid metabolism in salmon at two life stages (2.5g larvae and 10g juvenile) fed contrasting diets with vegetable versus fish oil. In pyloric caeca of vegetable oil fed salmon, we found strong up-regulation of cholesterol uptake, transport and biosynthesis pathways and, surprisingly, down-regulation of the fatty acid elongation and desaturation pathway. In liver, the pathways of cholesterol synthesis and fatty acid elongation and desaturation were both up-regulated. We also noticed differential plasticity in the ability of salmon to regulate lipid metabolism across life stages, with larvae responding more to dietary differences (Figure 2). This suggests that salmon larvae have a more sensitive response to vegetable oil diet than juveniles. Furthermore, dietary vegetable oil also had higher impact on fatty acids composition of larval than juvenile salmon, also suggesting that salmon larvae have less metabolic regulatory control when primed with vegetable oil diet.

Cysteamine pathway: a major taurine synthesizing pathway in common carp Cyprinus carpio

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Cloned cysteamine dioxygenase(ADO) in common carp (Cyprinus carpio) was consists of 790 nucleotide bases with 260 deduced amino acid. Phylogenetic analysis indicated that ADO in common carp is more advanced in evolution compared to Sinocyclocheilus rhinocerous. The hepatopacreas had a significantly high gene expression level among the organs examined.

To verify the taurine synthetic ability of common carp, cysteine and cysteamine(CHS) was injected to juvenile common carp at dose of 0, 0.25, 0.5 and 1mM and samples were collected at 0, 2, 4, 8, 12 and 24hrs. Injection of L-cysteine showed interaction and significantly affects levels of plasma taurine. High amount of L-cysteine significantly affected the plasma methionine level indicates the possibility that common carp has the ability to utilizes forward transsulfuration pathway. Both L-cysteine and CHS did not show interacting effect on hepatic total amino acid but significant increased in taurine after 24hrs was observed in 0.25mM injected samples. The interacting effect of injecting L-cysteine significantly affects cysteine dioxygenase 1(CDO1), cysteine sulfunic acid decarboxylase(CSD) and ADO gene expression but not cysteine dioxygenase 2(CDO2). CHS showed significant interacting effect on the gene expression of CDO1, CDO2 and ADO but not CSD.

The effect of dietary sulfur amino acid related compounds on content of sulfur amino acid related compound in hepatopancreas, gene expression of taurine synthesizing enzymes, somatostatin and growth hormone, growth and morphology was investigated. Common carp were fed either of eight diets supplemented with different level of 1.0 and 1.5%CHS, 1.5 and 3.0%cysteine, 1.0 and 1.5%methionine, and 0.5 and 1.0%taurine for 30days. The supplementation of 1.0 and 1.5%CHS caused growth retardation, myopathy and body deformity. All sulfur amino acids increased taurine deposition in the carcass (18.5-86.9 g/kg), and highest taurine content was observed in fish fed 1.5%CHS. The CDO1/2 tend to be down-regulated by supplementing cysteine and 0.5%taurine. Addition of cysteine, methionine and CHS down-regulate CSD. ADO was down-regulated by methionine and cysteine and 0.5% taurine. The somatostatin 14(SST14) was up-regulated by CHS. While insulin-like growth factor(IGF-1) was up-regulated by 1% taurine and cysteine. The present study suggests that CHS pathway is major taurine synthesizing pathway in common carp.

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Targeted gene expression panels and microbiota analysis provide insight into the effects of alternative production diet formulations on channel catfish nutritional physiology

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The present research evaluated targeted gene expression panels and microbiota analysis to provide greater insight into the effects of alternatively-sourced dietary ingredients on production indices, gut health, changes in the gut microbiota and genes involved in the regulation of appetite, growth, metabolism, and intestinal inflammation. Four dietary formulations were based primariliy on distinguishing protein sources: (D1-MFM) menhaden fishmeal (control) , (D2-MBM) porcine meat and bone meal, (D3-SBM) soybean meal, and (D4- CSM/CGM) cottonseed meal/corn germ meal, respectively, and fed to channel catfish for 12 weeks. Differences in feed conversion ratio (FCR), specific growth rate, feed intake, body condition, weight gain, proximal intestine histology, intestinal microbiota composition, and quantitative gene expression were analyzed. FCR was significantly ($P<0.05$) increased in D2 – 4 relative to D1-MFM; however, other production indices were unaffected by treatment. Dietary treatment also had no effect on intestinal histology ($P<0.05$). Effects of alternative dietary formulations on the gut microbiota were minimal, although when using Chao1, a significant effect of dietary treatment was detected ($P=0.0497$) on gut-associated microbiota richness estimates. D3-SBM caused diet-specific differences ($P<0.05$) in the expression of neuropeptide Y, peptide YY, and D2-MBM, D3-SBM, and D4-CSM/CGM resulted in differences in α-amylase, insulin receptor-a, glucose-6-phosphate-dehydrogenase , glucocorticoide receptor 1, and glucocorticoide receptor 2, relative to D1-MFM. These changes likely relate to differences in diet-mediated regulation of appetite and glucose metabolism, and perhaps the modulation of gut passage rate. By evaluating the molecular regulation of these pathways, as well as surveying the gut-associated microbiota, effects not detectable in short-term feeding trials may be elucidated which explain subtle differences in performance, such as FCR, as observed in the present study.

Regulating reproduction: RNA-seq analysis of variation in ovarian arachidonic acid levels in domesticated Penaeus monodon

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Recent works have demonstrated that current high-performance feeding regimes are not sufficient to sustain the high arachidonic acid (ARA) requirements for spawning in Penaeus monodon broodstock. Deficiencies in ARA content may impact egg production and maturation rates directly, or indirectly by limiting the production of downstream ARA-derived molecules with significant control over reproduction, such as prostaglandins. The current study investigated the availability, production and regulation of ARA and prostaglandin within fourth generation domesticated P. monodon broodstock (n = 19) fed an identical high-performance maturation diet. Broodstock demonstrated considerable variation in ovarian ARA content from 1.4 – 5.0%. To further inform biological conclusions drawn from biochemical data analyses, a reference P. monodon ovarian tissue transcriptome was assembled using 100 bp paired-end sequences generated on an Illumina HiSeq 2500. Differential gene expression analysis identified a total of 757 genes with greater than 2-fold expression change in response to variable ARA content. However, a paucity of genomic resources for P. monodon and crustaceans generally, resulted in only 19 genes that could be assigned functional gene ontology (2.5% of all genes identified). An alternative approach was undertaken, where a subset of the 40 most differentially expressed genes between the LOW and HIGH groups were manually annotated through a search of gene keywords in the UniProt database to assess putative gene function. A specific study of prostaglandin synthesis genes from this dataset indicated a significant positive correlation between ovarian ARA content and the expression of PmcPLA2 and PmCOX. Broodstock also demonstrated significant variation in the expression of PmCOX, PmPGFS, PmPGE1 and PmPGE3 as a function of varying levels of ovarian ARA. This study is the first demonstration of population level variation in ovarian ARA content, despite broodstock being fed identical high-performance maturation diets. Differential gene expression analysis demonstrates that variation in ARA has direct impact on the synthesis of key downstream prostaglandin synthesis genes, which have potent roles in broodstock egg production and maturation and elucidates a suite of currently uncharacterized genes for P. monodon.

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Hypomethylated CG islands of sirtuin promoters in gilthead sea bream (Sparus aurata)

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Fish exposed to sub-optimal rearing conditions are endangered for health and growth, and genes known as master regulators of energy sensing are of special relevance to disclose different types of metabolic disturbances and adaptive responses. This is the case of sirtuins (SIRTs), a conserved family of enzymes that couple protein deacetylation of histone and non-histone substrates with the energy status of the cell via the cellular NAD+/NADH ratio. In our previous studies, the seven SIRTs mammalian counterparts have been molecularly characterized in gilthead sea bream, revealing a ubiquitous gene expression that is tissue-specific for each SIRT. The different regulation of SIRT isotypes has also been proven in response to changes in nutrient deprivation or increased energy demand in fish strains with a high growth potentiality. To link changes in SIRT gene expression with epigenetic marks we aimed to underline the gene organization of gilthead sea bream SIRTs, focusing on the occurrence of CG islands (CGI) at the 5'-flanking region. In silico analysis by Blat searches in our genomic gilthead sea bream database (www.nutrigroup-iats-org/seabreamdb) revealed a conserved SIRT gene organization through the evolution with a variable number of exons that ranges from 3 in sirt4 to 16 in sirt2, while intron length varied considerably among SIRT isotypes and species. Intriguingly, CGIs were reported for the promoters of the nuclear sirt1 or mitochondrial sirt3, whereas those of sirt4-7 genes were defined as promoters with no evident CGIs. The methylation level of CGIs of sirt1 and sirt3 promoters and their possible correlation with changes in tissue gene expression was evaluated in liver, skeletal muscle and two intestine segments in two different models of improved growth and intestinal health due to: i) differences in genetic background and ii) dietary probiotic supplementation. In all cases, the methylation percentage of CGIs in sirt1 and sirt3 promoters did not differ between experimental conditions, showing an overall hypomethylation. These results point to the continuous expression level of sirt1 and sirt3 according to their important functions in energy sensing and metabolism. Further studies are underway to address the specific effects of temperature and age in SIRT CGI promoter methylation.

Micrornas Associate with Glucose Metabolism in Different Organs of Blunt Snout Bream (Megalobrama Amblycephala)

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Blunt snout bream (Megalobrama amblycephala) is a widely favored herbivorous fish species and is a frequentlyused fish model for studying the metabolism physiology. This study aimed to provide a comprehensive illustration of the mechanisms of a high-starch diet (HSD) induced lipid metabolic disorder by identifying microRNAs (miRNAs) controlled pathways in glucose and lipid metabolism in fish using high-throughput sequencing technologies. Small RNA libraries derived from intestines, livers, and brains of HSD and normal-starch diet (NSD) treated M. amblycephala were sequenced and 79, 124 and 77 differentially expressed miRNAs (DEMs) in intestines, livers, and brains of HSD treated fish were identified, respectively. Bioinformatics analyses showed that these DEMs targeted hundreds of predicted genes were enriched into metabolic pathways and biosynthetic processes, including peroxisome proliferator-activated receptor (PPAR), glycolysis/gluconeogenesis, and insulin signaling pathway. These analyses confirmed that miRNAs play crucial roles in glucose and lipid metabolism related to high wheat starch treatment. These results provide information on further investigation of a DEM-related mechanism dysregulated by a high carbohydrate diet.

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Dietary carbohydrate promotes de novo lipogenesis in barramundi (Lates calcarifer) as estimated using deuterated water (2H2O)

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In aquaculture, there is high interest in substituting fishmeal protein with carbohydrate-based ingredients such as vegetable starch. Farmed barramundi, as other carnivorous fish, when fed with starch-supplemented diets tend to deposit substantial amounts of lipid. However, is still unclear whether this overall increased adiposity and can be directly attributed to dietary carbohydrates. In order to investigate how carnivorous fish regulate the nutritional cues for these ingredients, and how they metabolically affect lipid retention and synthesis, we determined lipogenic fluxes in barramundi using deuterated water (2H2O) as metabolic tracer.

Barramundi (Lates calcarifer) were fed with two isolipidic diets: a high protein diet (Protein), and a diet with 30% pregelatinized starch (Starch). Fish were transferred to saltwater enriched with 2H2O (~3.5%). Lipogenic fluxes for hepatic triglyceride (TAG) biosynthesis were estimated by measuring triglyceride TAG 2H-enrichment by 2H-NMR.

The fractional synthetic rate for hepatic TAG (= de novo lipogenesis) in barramundi fed with Starch diet was significantly higher than observed in in barramundi fed with Protein diet (0.62±0.08 vs. 0.35±0.10 % day-1, respectively). Hepatic TAG-bound glycerol synthetic rates were much higher, but with no significant differences between diets, highlighting the structural role as metabolic intermediary (Starch, 2.80±0.31 vs. Protein, 3.40±0.34 % day-1). Curiously, fractional synthetic rates for hepatic free fatty acids were not statistically different.

Dietary starch did promote the storage of energy in the form of hepatic TAG as a results of increased de novo lipogenesis. However, we speculate that free fatty acids and glycerol, as part of structural functioning, remain unaltered by dietary starch.

COMPARATIVE ANALYSIS OF DIGESTIVE ENZYMES OF SYMPATRIC PAIR OF WHITEFISHES (ALTAI REGION, RUSSIA)

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Coregonus lavaretus – is a widely spread species of whitefishes in the north hemisphere that may form different sympatric pairs in lakes. This group of whitefish is very important object for Russian aquaculture industry characterized by high quality of fillet and caviar. One of such pair inhabits in Lake Teletskoye that is formed by Coregonus lavaretus pidshian (zoobentivorous) and Coregonus pravdinellus (zooplanktivorous) with less\more than 30 gill rakers on the first brachial arch respectively. These fish are very interesting model to study the sympatric evolution process. The aim of the study was to compare various parameters such as activity, parameters from Michaelis-Menten equation, pH and temperature optimums of the most important groups of digestive enzymes (proteases, amylases, and lipase) in fish gut.

Fish were caught in Lake Teletskoye (51°79'N; 87°26'E) by nets. Then, fish were dissected and their guts extracted and frozen in liquid nitrogen until their analysis. The proximate composition (concentration of protein, sugars, and lipids) of dominant food items was estimated. The activities of α-amylase, lipase, non-specific esterase, total alkaline proteases, trypsin, chymotrypsin, carboxypeptidase A and B, aminopeptidase and alkaline phosphatase were assayed. The number of alkaline protease bands on SDS-PAGE electrophoresis with casein as a substrate was assayed. Km, Vm from Michaelis-Menten equation, temperature and pH optimums for studied enzymes will be also presented as well as the aged- and circadian-dependences of activities.

The concentration of protein was similar between different food items. The activity of all studied enzymes had the similar trends along all parts of intestine of whitefishes, but the level of activities depended on the species considered. Thus, the activities of alkaline proteases and alpha-amylase were higher in the anterior and middle intestine of C.l. pidshian. The number of bands of alkaline proteases in the intestine between fish was similar. In general, the studied parameters of digestive enzymes (distribution of activities along the gut, zymogram profiles of alkaline protease, et.) are similar. It could be explained by relatively young age of this pair of whitefishes in terms of evolutionary time. This work was supported by the Russian Science Foundation, project no. 17-74-10071.